

Amendments to the Claims

Claims 1-7 and 17-47 were previously cancelled. With this amendment, please amend claims 8, 48, 55, and 60; cancel claim 10; and add new claims 70-75, as indicated below:

Claims 1-7. Cancelled.

Claim 8. (currently amended) A method of directing differentiation of human embryonic cells to a specific cell type, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~ providing chemically dissociated cultured human embryonic stem (hES) cells;

b. aggregating the dissociated hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;

[[b]]c. dissociating the embryoid bodies to provide dissociated embryonic cells;

[[c]]d. culturing said dissociated embryonic cells as a monolayer; and

e. exposing said ~~dissociated embryonic cells derived from the embryoid bodies~~ embryonic cells to at least one exogenous factor for an effective period of time; and

d. ~~causing directed~~ to direct differentiation of said dissociated embryonic cells to ~~form the~~ a specific cell type comprising a marker for terminally differentiated cells of the specific cell type.

Claim 9. (original) A method according to claim 8, wherein the embryoid bodies are formed in a suspension culture,

Claim 10. (cancel)

Claim 11. (original) A method according to claim 8, wherein the exogenous factor is a growth factor.

Claim 12. (original) A method according to claim 8, wherein the exogenous factor is an interleukin.

Claim 13. (original) A method according to claim 11, wherein the exogenous factor is nerve growth factor.

Claim 14. (original) A method according to claim 8, wherein the exogenous factor is retinoic acid.

Claim 15. (original) A method according to claim 8, wherein the differentiated cells are neuronal cell type.

Claim 16. (original) A method according to claim 15, wherein the differentiated cells have neuronal processes.

Claims 17-47. Cancelled.

Claim 48. (currently amended) A method of directing differentiation of human embryonic cells to human ectoderm cells, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~ providing chemically dissociated cultured human embryonic stem (hES) cells;

b. aggregating the dissociated hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;

[[b]]c. dissociating the embryoid bodies to provide dissociated embryonic cells;

[[c]]d. culturing said dissociated embryonic cells as a monolayer; and

~~e.~~ exposing said ~~dissociated embryonic cells derived from the embryoid bodies~~ embryonic cells to at least one exogenous factor for an effective period of time; and

~~d.~~ ~~causing directed~~ to direct differentiation of said dissociated embryonic cells to ~~form~~ human ectoderm cells comprising a marker for terminally differentiated human ectoderm cells.

Claim 49. (withdrawn) A method according to claim 48, wherein, in causing, said embryonic cells form human epidermal skin cells.

Claim 50. (currently amended) A method according to claim 49, wherein, in exposing, the at least one exogenous factor includes EGF.

Claim 51. (currently amended) A method according to claim 48, wherein, in ~~causing~~ exposing, said embryonic cells form human brain cells.

Claim 52. (previously presented) A method according to claim 51, wherein, in exposing, the at least one exogenous factor includes at least one of RA and NGF.

Claim 53. (withdrawn-currently amended) A method according to claim 48, wherein, in ~~causing~~ exposing, said embryonic cells form human adrenal cells.

Claim 54. (previously presented) A method according to claim 53, wherein, in exposing, the at least one exogenous factor includes RA.

Claim 55. (currently amended) A method of directing differentiation of human embryonic cells to human endoderm cells, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~ providing chemically dissociated human embryonic stem (hES) cells;

b. aggregating the dissociated hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;

[[b]]c. dissociating the embryoid bodies to provide dissociated embryonic cells;

[[c]]d. culturing said dissociated embryonic cells as a monolayer; and

e. exposing said ~~dissociated embryonic cells derived from the embryoid bodies~~ embryonic cells to at least one exogenous factor for an effective period of time; and

d. ~~causing directed to direct~~ differentiation of said dissociated embryonic cells to form human endoderm cells comprising a marker for terminally differentiated human endoderm cells.

Claim 56. (withdrawn-currently amended) A method according to claim 55, wherein, in ~~causing~~exposing, said embryonic cells form human liver cells.

Claim 57. (previously presented) A method according to claim 56, wherein, in exposing, the at least one exogenous factor includes at least one of HGF and NGF.

Claim 58. (withdrawn-currently amended) A method according to claim 55, wherein, in ~~causing~~exposing, said embryonic cells form human pancreatic cells.

Claim 59. (previously presented) A method according to claim 58, wherein, in exposing, the at least one exogenous factor includes at least one of HGF and NGF.

Claim 60. (currently amended) A method of directing differentiation of human embryonic cells to human mesoderm cells, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~providing chemically dissociated human embryonic stem (hES) cells;

b. aggregating the hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;

~~[[b]]c. dissociating the embryoid bodies to provide dissociated embryonic cells;~~
~~[[c]]d. culturing said dissociated embryonic cells as a monolayer; and~~
d. exposing said ~~dissociated embryonic cells derived from the embryoid~~
~~bodies~~ embryonic cells to at least one exogenous factor for an effective period of time;
and
~~d. causing directed~~ direct differentiation of said dissociated embryonic
cells to ~~form~~ human mesoderm cells comprising a marker for terminally
differentiated human mesoderm cells.

Claim 61. (withdrawn-currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human chondrocytes.

Claim 62. (previously presented) A method according to claim 61, wherein, in exposing, the at least one exogenous factor includes BMP-4.

Claim 63. (withdrawn-currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human kidney cells.

Claim 64. (withdrawn-currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human Mullerian duct cells.

Claim 65. (currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human blood cells.

Claim 66. (withdrawn-currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human heart muscle cells.

Claim 67. (previously presented) A method according to claim 66, wherein, in exposing, the at least one exogenous factor includes at least one of TGF- β and activin-A.

Claim 68. (withdrawn-currently amended) A method according to claim 60, wherein, in ~~causing~~exposing, said embryonic cells form human skeletal muscle cells.

Claim 69. (previously presented) A method according to claim 68, wherein, in exposing, the at least one exogenous factor includes at least one of TGF- β and activin-A.

70. (new) A method of directing differentiation of human embryonic cells to human neuronal cells comprising:

- a. providing chemically dissociated human embryonic stem (hES) cells;
- b. aggregating the dissociated hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;
- c. dissociating the embryoid bodies to provide dissociated embryonic cells;
- d. culturing said dissociated embryonic cells as a monolayer; and
- e. exposing said embryonic cells to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to neuronal cells.

71. (new) A method of directing differentiation of human embryonic cells to human muscle cells comprising:

- a. providing chemically dissociated human embryonic stem (hES) cells
- b. aggregating the hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;
- c. dissociating the embryoid bodies to provide dissociated embryonic cells;
- d. culturing said dissociated embryonic cells as a monolayer; and
- e. exposing said monolayer to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to muscle cells.

72. (new) A method according to claim 71, wherein the muscle cells are cardiomyocytes.

73. (new) A method of directing differentiation of human embryonic cells to human pancreatic cells comprising:

- a. providing chemically dissociated human embryonic stem (hES) cells;
- b. aggregating the hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells in the embryoid bodies undergo initial differentiation;
- c. dissociating the embryoid bodies to provide dissociated embryonic cells;
- d. culturing said dissociated embryonic cells as a monolayer; and
- e. exposing said embryonic cells to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to form pancreatic cells.

74. (new) A method of making human embryonic bodies from human embryonic stem cells comprising:

- a. providing chemically dissociated human embryonic stem (hES) cells;
- b. aggregating the hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation.

75. (new) A method of making human embryonic cells from human embryonic bodies comprising:

- a. providing chemically dissociated human embryonic stem (hES) cells;
- b. aggregating the hES cells in suspension in media lacking LIF and bFGF to form embryoid bodies, wherein the cells of the embryoid bodies undergo initial differentiation;
- c. dissociating the embryoid bodies to provide dissociated embryonic cells; and
- d. culturing said dissociated embryonic cells.